MECHANISMS ASSOCIATED WITH DISEASE CONTROL BY ORGANIC SOIL AMENDMENTS

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Introduction

The fungus Verticillium dahliae (kleb.) is a wilt pathogen of a variety of crops in Ontario and worldwide (1). Verticillium wilt of potato or "early dying syndrome!' causes pre-mature senescence. Infection occurs through roots via microsclerotia (MS) that overwinter in soil and plant residue. Verticillium wilt is difficult to manage because of limited success with crop rotations, and slow development of new resistant cultivars. Other than fumigation of soil, there are no other available chemical control options.

Amendments high in organic N (N>8%) such as blood and fish meal when added to soil (1% w/w) have been shown to inhibit Verticillium infection of tomato (2). Other soil-borne pathogens have been reported to be controlled by high organic N amendments including: various oil seed meals (3, 4); chitin (5); and chitin-urea (6). Studies by our laboratory with early dying syndrome confirmed Whilhelm's finding and extended it meat and bone meal (MBM) in a sand soil (7). Further, it was shown that MS suspended above amended soil lost viability suggesting a toxic volatile was involved. Within I week of MBM addition, soil pH also rose above 8.5 and present was an odor of ammonia (NH_3). Surprisingly, MBM to a loam soil (2% w/w) failed to increase soil pH, control MS and have an odor of NH_3. Together, NH_3 generation was implicated in controlling MS.

The objectives of this study were to: a) determine if NH_3 accumulation in soil is related to control of MS; b) show NH_3 to be toxic to MS; and c) determine the factor(s) controlling NH_3 accumulation in soil. To answer these objects we contrasted NH3 accumulation, MS viability, and parameters in soil that MBM controls (labelled responsive) and does not control MS (unresponsive).

Methods

Bioassay: MS viability in soil by this bioassay has been shown representative of potato infection incidence in field and greenhouse experiments by our laboratory. The bioassay is similar to (8) with modifications to increase the number samples handled. Briefly, MS were raised in sterile culture, at 3 weeks MS obtained by screening, 15mg of MS added to 5g silica, mixed, 15mg of the suspension added to a nylon bag and placed in soil. Soil was obtained from 3 locations in a field, mixed dried, screened, amended (w/w), and mixed. To tubes, 20 g amended soil was added. Water was added to 0.33 bar tension in soil to standardize moisture availability to microorganisms (9). At intervals, bags were retrieved with 3 replicates per sample date. MS viability was determined by plating contents of the bag onto selective media and determination of germination at 2 weeks.

N **Transformations:** Total (NH_3+NH_4^+), $N0_2^-$ and $N0_3^-$ in cold water extracts were determined by ion exchange and conductivity using an Ion Chromatograph. NH_3 was calculated as the fraction of total ($NH_3^+NH_4^+$) in solution depending upon soil pH and incubation temperature (10).

Toxicity in Media: NH_3 toxicity to MS was determined by generation of NH_3 in solid media from NH_4Cl at increasing pH. The concentration of NH_3 was calculated as previously described. Activity of NH_3 in solution was held constant for various NH_3 concentrations by adjustment of ionic strength with NaCl addition. Immediately upon cooling of media 50 MS were plated and the petri dishes wrapped to reduce volatilization losses.

Results and Discussion

MBM was added to a responsive sand and unresponsive loam soil and contrasted for NH_3 accumulation and MS viability (Fig. 1). With the sand, 2% MBM decreased MS viability to near 0 by day 4 and remained <10% to the end of the study. This contrasted with the loam in which MS viability remained >60%. In sand, a rapid decline in MS viability corresponded with an increase in soil pH to 9 and accumulation of NH_3. Continued survival of MS in the loam corresponded with low NH_3 and high $N0_3^-$ accumulation. 1% MBM to the sand resulted in a gradual decline of MS viability to 0. This decline in the sand coincided with colonization of MS by a particular fungus and not NH_3 accumulation. With 1% MBM to the responsive sand, an alternate mechanism of control of MS not due to NH_3 toxicity was indicated.

Association between NH_3 accumulation and rapid MS decline lead to testing toxicity of NH_3. Increasing the NH_3 concentration in media prevented MS germination (Fig. 2). Similarly, NH_3 toxicity in the unresponsive loam was induced by urea-N equivalent to 2% MBM and the nitrification inhibitor dicyandiamide (DCD; Fig. 2). The $LD_{100}$from the soil assay agrees with results of the previous study in which MS viability in the sand was near 0 at 68 mg NH_3-N kg^{-1} soil. Together, these studies confirm MS survival in the unresponsive loam with MBM due to the lack of NH_3 accumulation.

To determine the factor(s) controlling NH_3 accumulation in soil, MBM (2% w/w) was added to 12 soils. Selected soil properties potentially controlling NH_3 accumulation were followed: physical (texture, organic C); chemical (NH_3, $N0_2^-$, $N0_3^-$, buffering capacity, cation exchange capacity, electrical conductivity); and biological (total bacteria, fungi, ammonifying bacteria, ammonifying fungi, proteolytic bacteria, soil respiration). NH_3 accumulated in 4 of the 12 soils, effectively preventing MS viability. The parameter most related to NH_3 accumulation in soil was organic C (Fig. 3). The relationship between peak NH_3 and organic C was linear (r^2=0.95) and suggests to control MS following 2% MBM organic C must be <1.7%. Retention of NH_3 onto organic matter has been well documented for fertilizer N (11) but this study suggests it can be from organic sources as well.

This research is timely because the Potato Association of America has rated early dying syndrome the second most important disease and the first most important yield constraint to potato (12). Industry and growers have for the past few decades relied upon fumigants such as Methyl Bromide. We hope this research will provide the basis to an alternative approach. Studies in our lab have found the benefits of using MBM compared to fumigants, considerable. They include: a) increase in soil microbial populations and diversity; b) broad spectrum control of nematodes, weeds, other soil-borne fungi and bacteria; c) increase in soil fertility; and d) provide residual control for several years.

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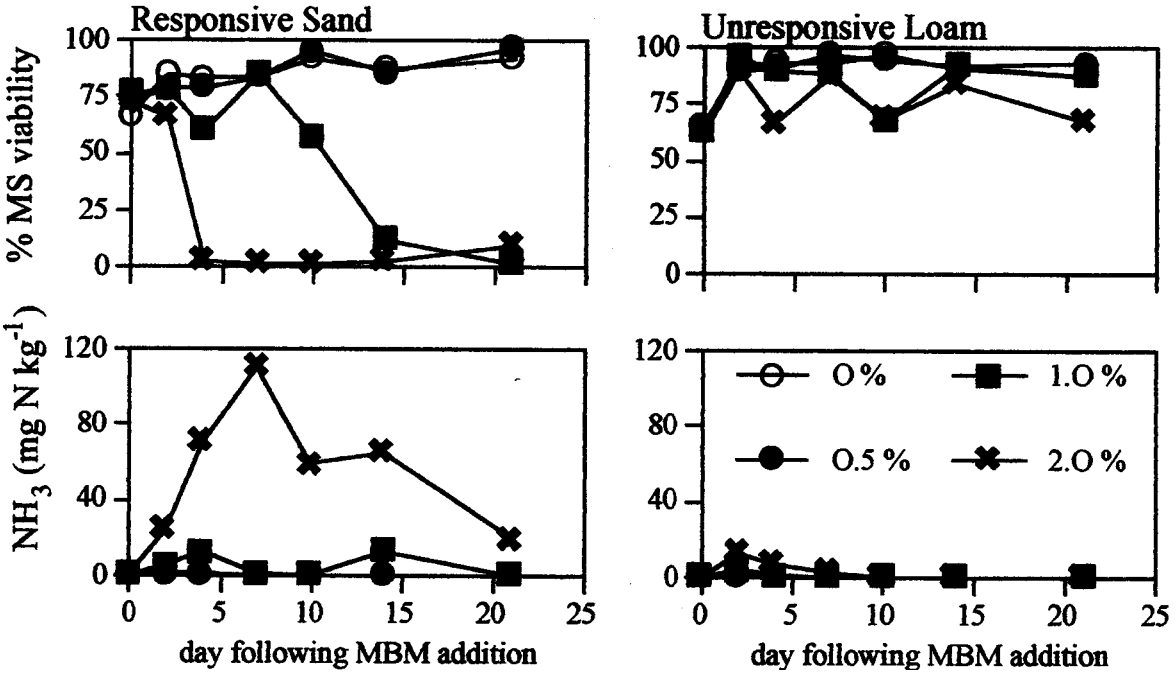

Fig. 1. MS viability and NH_3 content with rates of MBM (w/w) to a responsive and unresponsive soil.

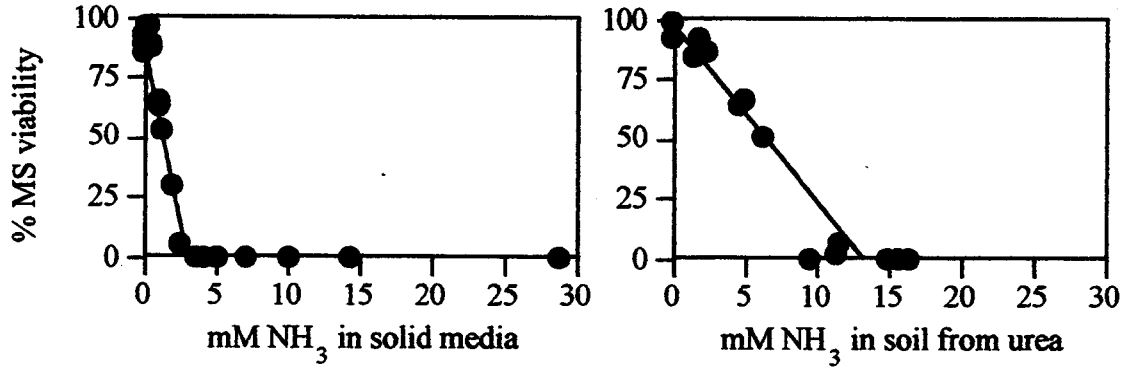

Fig. 2. MS viability to NH_3 in solid culture media and in unresponsive soil with urea and DCD.

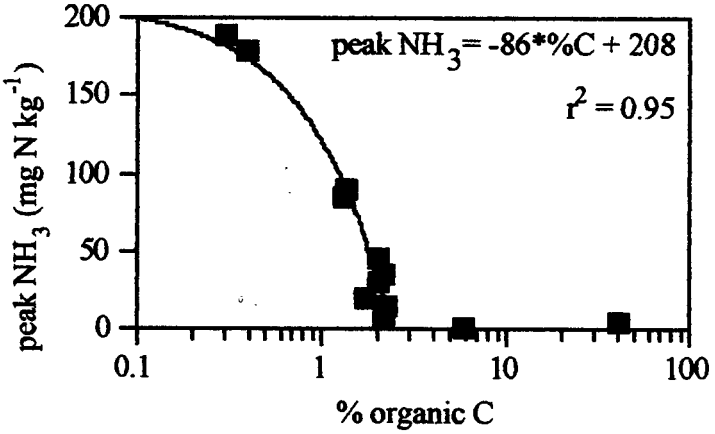

Fig. 3. Peak NH_3 content of soil versus organic C in 12 soils amended with MBM (2% w/w).